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Sex determination in the haplodiploid wasp *Nasonia vitripennis* (Hymenoptera Chalcidoidea)

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Review

Sex determination in the haplodiploid wasp *Nasonia vitripennis* (Hymenoptera: Chalcidoidea): A critical consideration of models and evidence

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Abstract

Sex determining mechanisms are highly diverse. Like all Hymenoptera, the parasitic wasp *Nasonia vitripennis* reproduces by haplodiploidy: males are haploid and females are diploid. Sex in *Nasonia* is not determined by complementary alleles at sex loci. Evidence for several alternative models is considered. Recent studies on a polyploid and a gynandromorphic mutant strain point to a maternal product that is balanced against the number of chromosomal complements in the zygote and a parent-specific (imprinting) effect. Research is now focused on the molecular details of sex determination in *Nasonia*.

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Keywords: Genomic imprinting; Hymenoptera; *Nasonia*; Polyploidy; Sex determination

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1. Introduction

An intriguing question is to what extent sex determining mechanisms are conserved during evolution. The fact that almost all multicellular sexual species reproduce with either separate

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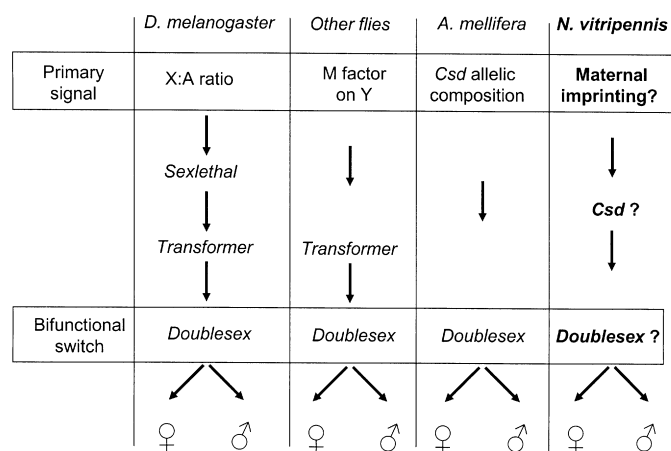


Fig. 1. Sex determination cascades in Diptera and Hymenoptera. Evolutionary conservation decreases from the bottom upwards. A: autosome sets, X: X-chromosome, Y: Y-chromosome, M: male determining factor, *Csd*: complementary sex determiner.

or combined male and female function, may suggest similar underlying genetic mechanisms for sexual differentiation. However, sex-determining mechanisms vary considerably and change rapidly in the course of evolution [1]. After elucidation of the genetics of sex determination in *Drosophila melanogaster* and *Caenorhabditis elegans* at the end of the last century [2], data have started to accumulate on the genetic regulation of sex determination in a large number of organisms. This allows for a more thorough consideration of the evolution of sex-determining mechanisms. An illustrative example is sex determination in a number of insects [3–16]. Consistent with Wilkins [17], these studies reveal evolutionary conservation at the basis of gene cascades, but divergence at the level of primary signals (Fig. 1).

There is a long-standing interest in sex determination of hymenopteran insects (ants, bees, sawflies and wasps) due to their haplodiploid mode of sex determination and the absence of heteromorphic sex chromosomes. Arrhenotoky is the most prevalent mode of reproduction among Hymenoptera: males are haploid and develop parthenogenetically from unfertilized eggs, whereas females are diploid and develop from fertilized eggs (Fig. 2). Sex determination is somehow triggered by the number of chromosome sets present in the embryo, but still little is understood about the molecular regulation. For over 60 years, it has been known that different sex determining mechanisms exist within the Hymenoptera [18]. Under complementary sex determination (CSD), gender is genetically determined by a single locus with multiple alleles: individuals that are heterozygous at this locus develop into females, whereas hemizygotes and homozygotes develop into haploid and diploid males, respectively [18]. This mode of sex determination has now been shown for more than 60 species [19]. It is considered ancestral although very few species from the basal taxonomic groups have been tested for CSD (Fig. 3) [20]. The *csd* locus was recently identified and cloned for the honey bee [8]. However, sex determination in some groups, such as the large parasitoid wasp group Chalcidoidea, can clearly not be explained by CSD because homozygous diploids develop into females nevertheless.

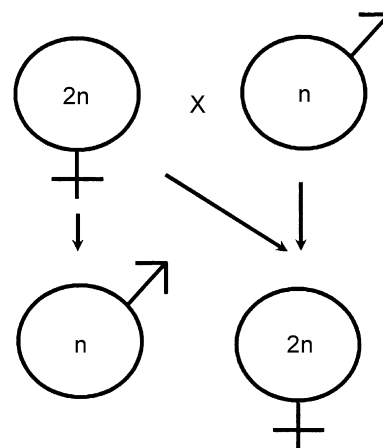


Fig. 2. Haplodiploid sex determination. Females are diploid and produce haploid eggs. Males are haploid and produce haploid sperm. Unfertilized eggs develop into haploid males and fertilized eggs into diploid females.

The parasitic wasp *Nasonia vitripennis* has been extensively studied genetically and is rapidly being recognized as a model system in evolutionary and developmental biology [21–25]. It has been known for a long time that its sex determination is not governed by CSD [26,27], but for many years progress has been made in elucidating its mode of sex determination [28]. Recently, several studies reported on the genetics of sex determination in *N. vitripennis* [29–32]. In this paper, we compile and interpret the currently available genetic data in relation to the proposed alternative models to CSD.

2. Mutant strains

Many of the discussions about sex determination in *N. vitripennis* have been prompted by attempts to accommodate observations on the ploidy level and sex of aberrant individuals. Whiting [33] reported spontaneous mutations to polyploidy in his stock cultures and one such strain has been maintained ever since. It has been used by Dobson and Tanouye [34] and Beukeboom and Kamping [29]. Triploid females have low fecundity and produce both haploid and diploid eggs, both of which normally develop into males if unfertilized, but into females if fertilized (Fig. 4). In the lab, it is possible to determine the ploidy level by using two different recessive eye-colour mutations, *scarlet* and *oyster* that do not recombine. Homozygosity at the mutant allele at either one of these loci results in a deviation from phenotypically “wildtype” (purple) eyes [35]. Diploid males are fully fertile. They produce diploid sperm mitotically, indicating that ploidy level does not determine the mechanism of spermatogenesis (meiotic or mitotic). They are mated to diploid homozygous *scarlet* females to re-obtain triploid females that carry two chromosome sets of the father and one set of the mother (see Fig. 4). Hence, it is important to realise that diploid males that are known from *N. vitripennis* do not arise from homozygosity under CSD (see below) but from unfertilized diploid eggs.

Males from some natural populations carry the paternal sex ratio (PSR) distorter [36]. PSR is a supernumerary chromosome

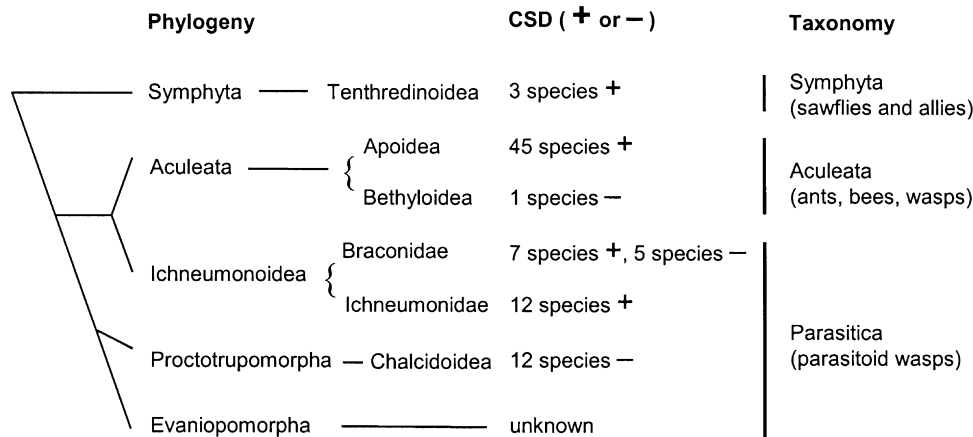


Fig. 3. Phylogenetic distribution of complementary sex determination. Redrawn from Cook and Crozier [20] with additional data from Van Wilgenburg et al. [19]. Presence and absence of CSD is indicated by (+) and (-), respectively.

that is transmitted through sperm and causes condensation and loss of the paternal chromosomes in fertilized eggs, itself surviving [37]. Because of haplodiploidy, PSR transforms fertilized diploid eggs that would normally develop into females, into haploid eggs that develop into males. These males only carry maternal chromosomes, as do normal haploid males that develop from unfertilized eggs, but they additionally receive the PSR chromosome from their father. The PSR chromosome appears devoid of any structural genes [38].

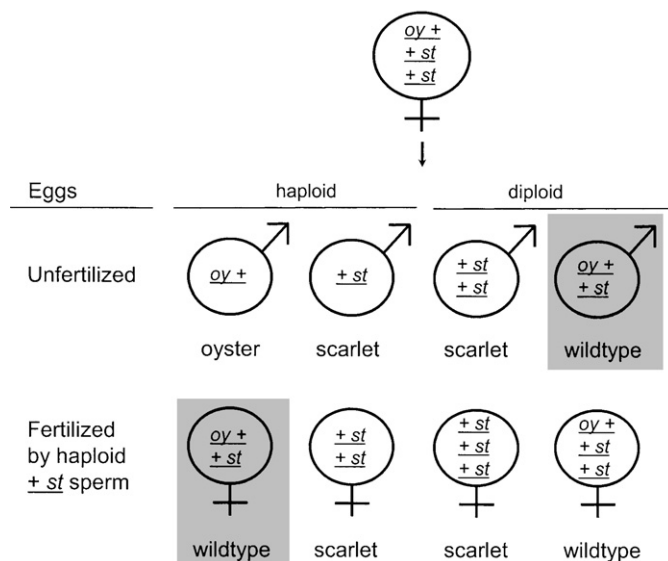


Fig. 4. The polyploid mutant strain of *Nasonia*. Recessive eye-colour mutations, *oyster* (*oy*) and *scarlet* (*st*) on a non-recombining locus are used to distinguish ploidy levels. Virgin triploid females lay haploid and diploid eggs which develop into males if unfertilized. Fertilization of haploid and diploid eggs by haploid *st* sperm results in female development indicating a parental sex-specific effect on sex determination (compare *oy* +/+ *st* unfertilized eggs versus *oy* +/+ *st* fertilized eggs in grey compartments). Distinction between haploid (*oyster*) and diploid (wildtype) males is possible, but haploid and diploid *scarlet* males cannot be distinguished. Female ploidy level is determined by offspring number (triploids have low fecundity) and segregation of eye colours among their offspring. Genotypes are indicated inside and phenotypes below the male and female symbols.

Beukeboom et al. [31] and Kamping et al. [32] report on a natural *N. vitripennis* strain that produces gynandromorphs (i.e. female–male mosaics) and uniparental females from haploid eggs that would normally develop into males. Genetic experiments showed that the trait inherits as a major maternal effect locus, called *gynI*, located on chromosome IV. The occurrence of haploid females is inconsistent with any of the proposed models of sex determination in *Nasonia* as will be discussed below.

3. Sex determination models

3.1. Single-locus complementary sex determination (sl-CSD)

Under sl-CSD sex is determined by a single locus with multiple alleles. Heterozygotes develop into females, while hemizygotes and homozygotes become males [18]. Whiting developed this model based upon his studies with the braconid wasp *Bracon hebetor*. However, this model has been discarded for the superfamily Chalcidoidea based upon the absence of diploid males in long-term laboratory cultures, where an increase of homozygosity at the sex locus should occur due to loss of sex allele variation. It has only been formally rejected for the chalcidoid *N. vitripennis* through controlled inbred crosses for several generations [27].

3.2. Multi-locus complementary sex determination (ml-CSD).

Crozier [39] following Snell [40] extended the sl-CSD model to multiple loci with multiple alleles. Individuals that are heterozygous for at least one of these loci become females. To date, ml-CSD has never been convincingly documented for any hymenopteran, although some recent support was found [41]. It is inconsistent with some forms of thelytokous reproduction in which diploid females develop parthenogenetically from unfertilized eggs. For example, thelytoky can be caused by intracellular *Wolbachia* bacteria that induce gamete duplica-

tion in haploid eggs [42]. The resulting offspring are completely homozygous and should therefore develop into males [19,43]. Since *N. vitripennis* can be inbred for many generations without resulting diploid males or reduced progeny sizes, ml-CSD is also rendered unlikely.

3.3. Fertilization sex determination (FSD)

Whiting [33] suggested that fertilization determines the sex of an embryo, i.e. unfertilized eggs become males, and fertilized eggs become females. It was rejected for *Nasonia* based on crosses with males that carry the PSR chromosome [34,44]. PSR destroys the paternal genome in fertilized eggs that would normally develop into females transforming them into haploid eggs that develop into males. The FSD model predicts that PSR fertilized eggs will develop into haploid females rather than into haploid males. FSD is also inconsistent with thelytokous hymenopterans in which females produce diploid daughters from unfertilized eggs.

3.4. Genic balance sex determination (GBSD).

The GBSD model [45] predicts that sex is determined by a balance between non-cumulative male (*M*) and cumulative female (*F*) loci. In haploids, *M* is stronger than *F* resulting in males, whereas in diploids *M* is outweighed by $2F$ resulting in females. This model cannot explain the existence of diploid males in the polyploid mutant of *N. vitripennis* because the *F* loci should outweigh the *M* loci ($2F > M$) and lead to femaleness. In an attempt to explain the existence of diploid males with the GBSD model, Stouthamer and Kazmer [46] hypothesized that diploid males carry one mutated *F* locus (i.e. they are functionally haploid). However, this suggestion could also be rejected, because it predicts that all triploid females would produce one-third of daughters (those with the two non-mutated *F* loci) among their diploid offspring, which is not true [29].

3.5. Maternal effect sex determination (MESD)

Cook [43] following Crozier [47] proposed that sex is determined by a balance between a cytoplasmic and a nuclear component. The cytoplasmic component is a maternal gene product put into the egg by the female during oogenesis, but this product is not a heritable cytoplasmic element (e.g. mitochondria). Examples of such maternal effects are *daughterless* in *Drosophila melanogaster* and the feminizing *F*-factor in *Musca domestica* [48,49]. In haploid embryos the cytoplasmic component (*MP* for maternal product) is masculinizing, but in diploid embryos it is outweighed by the nuclear genes (*C* for number of complements) resulting in female development. Dobson and Tanouye [34] rejected this model based on their observation that diploids can be male in the polyploid strain. Their interpretation of the MESD model is that the cytoplasmic component in diploid eggs is outweighed by two sets of chromosomes resulting in females ($2MP < 2C$), but this is not necessarily true. A better interpretation of this model is that diploid eggs of triploid females receive a triple dosage of the cytoplasmic component,

i.e. there are three copies of the gene transcribing the maternal product. Hence, the cytoplasmic component is not outweighed by two sets of chromosomes ($3MP > 2C$) and this would result in diploid males, which was consistent with the data at that time. Therefore, Dobson and Tanouye's rejection of the MESD model was premature.

3.6. Genomic imprinting sex determination (GISD)

An observation inconsistent with the MESD model is that haploid eggs of triploid females develop into females when fertilized by haploid sperm, i.e. $3MP > 2C$ should lead to males (Fig. 4). Dobson and Tanouye [34] observed this but failed to recognize its significance. Both diploid unfertilized eggs and haploid fertilized eggs of triploid females contain two chromosome sets, the only difference being that the first has both sets of maternal origin, whereas the latter contain one set of each parent. Since these two types of eggs give rise to different sexes, it implies that a paternally inherited complement is functionally different from a maternal one, i.e. there is a parental sex-specific effect on sex determination. This observation formed the basis for the genomic imprinting sex determination (GISD) hypothesis [28,50] which predicts that a paternally inherited set of chromosomes is required for female development. The original version views female development as resulting from binding of a product (*P*) present in the egg or zygote to a nuclear locus (*S*) when it is not imprinted. Females imprint *S* which prevents *P* from binding and causes haploid eggs to develop into males. Males do not imprint *S* and provide a binding site for *P*, allowing female development in fertilized eggs.

The GISD model is similar to the MESD model in many of its predictions about the offspring sex in crosses using the polyploid mutant and PSR strains. Dobson and Tanouye [34] accepted the GISD model by rejection of all alternatives, but they falsely discarded the MESD model (see above). Recently, new data have become available concerning the sex of individuals that carry particular combinations of maternally and paternally derived chromosome complements. We will now consider how the MESD and GISD model can or cannot accommodate these data.

4. Recent progress

Recent studies provide new information about sex determination in *N. vitripennis*. Beukeboom and Kamping [29] performed additional crosses with the polyploid mutant and found that triploid females can produce diploid daughters parthenogenetically. Some individuals among the offspring of about 2% of triploid females developed into gynandromorphs or fertile females rather than males from unfertilized diploid eggs. Segregation of non-functional loci was ruled out as possible explanation, but instead, a rare epigenetic event appeared responsible for these results. Because both the MESD and the GISD model predict that unfertilized diploid eggs become male, modifications have to be invoked to accommodate occasional parthenogenetic female production. Under MESD such females can be viewed as descendants of eggs lacking a full triple dosage

of maternal product, shifting the maternal product-chromosome complement balance towards females. GISD would explain these individuals by occasional failure to imprint one or both chromosome sets by the mother.

We [31,32] studied a natural *N. vitripennis* strain that produces gynandromorphs and females from haploid eggs. Our results again show that females can develop from eggs that do not receive a paternal chromosome complement, as required under the GISD model. The data are also in conflict with the MESD model which predicts that in haploid eggs two doses of maternal product outweigh one chromosome complement ($2MP > 1C$) leading to males. One has to assume that this gynandromorphic strain either has a non-functional maternal imprinting mechanism or a mutant maternal effect locus, which prevents masculinization of the zygote.

Kamping and Beukeboom (unpublished) performed crosses between the polyploid mutant and the gynandromorphic strain. Diploid males were crossed with females of the gynandromorphic strain yielding triploid females with two chromosome complements of the polyploid strain and one set of the gynandromorphic strain in the gynandromorphic strain's cytoplasm. These females produced a high frequency (17%) of gynandromorphs and daughters from predominantly diploid unfertilized eggs. Hence, introduction of genetic material of the gynandromorphic strain in the polyploid strain, greatly increased the frequency of uniparental daughters. Again, these results can only be explained with the MESD and GISD models by invoking mutational changes in the maternal effect and imprinting locus, respectively, of the gynandromorphic strain.

Trent et al. [30] X-ray mutagenized wildtype haploid males and crossed them to homozygous recessive mutant females. They obtained rare diploid male offspring at frequencies similar to new mutations in eye-colour genes. Note that these diploid males are of biparental origin rather than uniparental in the polyploid strain. The authors could rule out a dominant loss-of-function mutation in subsequent crosses because the trait was not expressed in granddaughters of the diploid males. Unfortunately, they could not transmit the mutation through haploid males suggesting a pleiotropic lethal zygotic effect. The authors interpret their data in support of the genomic imprinting model. The biparental diploid males are explained by an imprinting defect in the irradiated paternal germ line generating an epigenetic lesion.

5. A new model

As discussed above both the MESD and GISD models require specific modifications to accommodate the new genetic data. We propose an adjusted model for sex determination in *N. vitripennis* that combines aspects of these two models. The model is called MEGISD for maternal effect genomic imprinting sex determination (Fig. 5). It proposes that sex in *N. vitripennis* is determined by a maternal effect gene (*msd* for maternal sex determiner) that imprints a zygotic sex determiner (*zsd*). Maternal imprinting of the *zsd* gene causes male development in the zygote. Being a maternal effect gene, *msd* is not active during spermatogenesis and the paternally inherited *zsd* gene is not imprinted, which results in female development. Since haploid

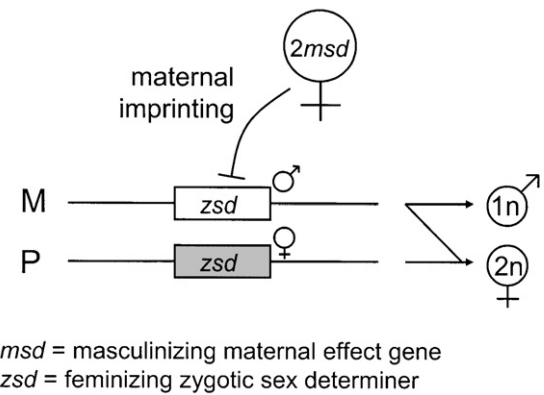


Fig. 5. The maternal effect genomic imprinting sex determination (MEGISD) model. A maternal effect gene (*msd*, two doses in diploid females) actively imprints a zygotic sex determiner (*zsd*) into a masculinizing state. The *msd* gene does not imprint in males and the paternally inherited *zsd* allele is feminizing. Unfertilized haploid eggs with only a maternal *zsd* develop into males and fertilized diploid eggs with a maternal and paternal *zsd* develop into females. Uniparental haploid females of the gynandromorphic strain are considered resulting from incomplete imprinting due to a mutation in the *msd* or *zsd* gene. The *msd* gene may be similar to *gyn1* of Kamping et al. [32] as discussed in the text.

eggs only contain a maternally derived *zsd* copy they develop into males, whereas diploid fertilized eggs in addition contain a non-imprinted paternal allele of *zsd* resulting in female development. Hence, the default sex in *Nasonia* is female and maternal silencing turns on the male pathway. The alternative of paternal activation of *zsd* by imprinting is less easy to reconcile with the maternal feminization that occurs in the gynandromorphic strain.

Although not necessarily, *msd* and *zsd* may be one and the same gene. Maternal regulation of zygotic activity is known from, for example, the *transformer* gene (*F*-factor) in *Musca domestica* and other flies [11,15]. However, the autoregulation of the *F*-factor in *M. domestica* is positive (maternal *F* turns on zygotic *F*) and feminizing, whereas in *Nasonia* it would be negative and masculinizing. The results of crossing experiments with the gynandromorphic strain by Kamping et al. [32] indicate that both the nucleus and cytoplasm are required for the expression of femaleness. This argues for not just an epigenetic modification of the *msd* gene, but also for the presence of an interactor of the *msd* gene in the egg cytoplasm. The *msd* locus can be considered analogous to the hypothesized *P* locus under the original GISD model [28]. In the original description of the GISD model the site and timing of synthesis of *P* was however not specified.

How can the MEGISD model explain the new data of the polyploid, PSR and gynandromorphic strain [29,32,34], as well as those of irradiated males [30]?

- (1) Diploid unfertilized eggs of a triploid female normally develop into males, but her haploid eggs that become fertilized develop into females. Triploid females transcribe three *msd* gene copies that silence both maternal *zsd* genes in their diploid eggs resulting in male development. Their haploid eggs contain one inactive *zsd* gene, but upon fertilization

inherit an active paternal copy resulting in female development.

- (2) Rare parthenogenetic development of daughters from diploid eggs of triploid females. This can be considered as a failure to maternally imprint one or both *zsd* genes. The very low frequency at which daughters occur supports the presence of an epigenetic mutation (or rare recombinational event) rather than a 'standard genetic' mutation.
- (3) Haploid females from the gynandromorphic strain. Similar to (2) one has to invoke an active maternally derived *zsd* gene that feminizes the embryo. This can be the result of a maternal imprinting failure by the *msd* gene or a modification in the imprintability of the *zsd* gene.
- (4) Frequent parthenogenetic development of daughters from diploid eggs that inherit the gynandromorphic strain's cytoplasm. This is again consistent with an altered imprinting effect of the *msd* gene in the gynandromorphic strain. It also points to a dosage effect since gynandromorphs and females develop predominantly from diploid rather than haploid eggs of the same mother. The balance in haploid eggs would be three doses of *msd** gene transcripts versus one maternally derived *zsd* gene, whereas in diploid eggs it would be three *msd** versus two maternal *zsd*, where the * refers to an (epi)genetic modification of the *msd* gene (Fig. 5).
- (5) Biparental diploid sons from irradiated males [30]. The easiest explanation for this observation is that the paternal *zsd* gene was mutagenized and rendered inactive, resulting in diploid eggs with a masculinizing maternal copy through imprinting and an inactive paternal copy through irradiation.
- (6) Androgenic males. In a frequently cited meeting abstract, Friedler and Ray [51] claim to have found haploid males from fertilized eggs with only the paternal chromosome complement after irradiation of females ("paternate" males). If true, it would be hard to explain with the MEGISD model, because haploid eggs with only an active paternal *zsd* gene should develop into females. It would therefore be worthwhile to repeat this experiment.
- (7) The *gyn1* gene. The easiest explanation for the maternal effect *gyn1* gene discovered by Kamping et al. [32] is that it corresponds to the proposed *msd* gene of the MEGISD model. *Gyn1* may be a hypomorph of the wildtype maternal effect gene with a reduced masculinizing effect. Alternatively, *gyn1* may interact with the *msd* gene resulting in a reduced maternal effect in the egg.

6. Sex determination and reproductive mode

6.1. Thelytoky

Many hymenopterans including several chalcidoids, but not *Nasonia*, reproduce by thelytoky. Under thelytoky, females develop from unfertilized eggs that become diploid by a variety of diploidy restoration mechanisms [52]. Most forms of thelytoky result in an increase of homozygosity [52] and with gamete duplication offspring become completely homozygous within a single generation. How can the MEGISD model explain this

since it relies on differential imprinting of paternally and maternally inherited genomes? One explanation is that thelytokous species have lost the ability of maternal imprinting of the *zsd* gene. Hence, they would always inherit an active feminizing copy. However, the observation that removal of *Wolbachia* by antibiotic curing [53] can revert such females to produce males from haploid eggs, argues for retention of imprinting. A more likely explanation is that the imprint of the *zsd* gene is not transferred onto the duplicated copy because duplication occurs after meiosis in the absence of the maternally active *msd* gene. Hence, the resulting embryo would have an inactive and a feminizing copy of the *zsd* gene resulting in female development. The attractiveness of this explanation is that evolution of thelytoky does not require a change in the sex determining mechanism, but rather only an alteration of meiosis.

6.2. Polyploidy

Mutations to polyploidy have arisen spontaneously in laboratory cultures of *N. vitripennis* several times [33], probably due to occasional non-disjunction in oogenesis. A mutation from diploidy to triploidy changes the relative doses of the *msd* and *zsd* genes from 2:1 (haploid eggs) to 3:2 (diploid eggs) in unfertilized eggs. A 3:2 dose may bring the sex switch closer to equality and therefore may cause occasional gynandromorphic and female development from diploid unfertilized eggs. Hence, the MEGISD model can explain sex determination in polyploid *Nasonia* without invoking mutations in sex determining genes. Therefore, mutations that led to polyploidy in *Nasonia* are likely to be independent of the mechanism of sex determination.

7. The *Nasonia* sex determining mechanism compared to other insects

The MEGISD model proposes the existence of a maternal effect gene and a zygotic sex-determining gene under regulation of a parental sex-specific imprint. Both phenomena are known from sex determining mechanisms in other insects. Maternal products have been found in several diptera, including *Drosophila melanogaster* [54], *Musca domestica* [49,55] and *Chrysomya rufifacies* [56]. Evidence for genomic imprinting involved in sex determination is however sparse and only known from X-chromosome elimination in sciarid flies [57,58].

To what extent homologous genes are involved in hymenopteran and dipteran sex determining mechanisms has been considered by several authors [5,7,10,17]. Some genes belonging to the *D. melanogaster* sex-determining cascade serve similar function in other Diptera, but others do not (Fig. 1). Genes at the bottom of the cascade, such as *doublesex*, are more conserved than genes higher up in the cascade, consistent with Wilkins' [17] hypothesis. *Doublesex*-like genes appear to be also present in *Nasonia* (C. Trent, unpublished, E. Verhulst, unpublished). To what extent *doublesex* is similarly regulated by *transformer* is currently being investigated in several organisms. Many questions remain about the genes involved in sex determination in *Nasonia*. The *csd* gene of the honeybee shows some homology to *transformer* [8], but whether a *transformer*-like

gene is involved in *Nasonia* sex determination is still speculative. Is the *csd* gene present in *Nasonia* and is it involved in sex determination? If yes, how is its complementary action overcome? Although present in other insects, *Sex-lethal* apparently only serves a function in sex determination and dosage compensation in drosophilids [2,5,7,12,16,59]. It is therefore expected that *Sex-lethal* will not be involved in *Nasonia* sex determination. What is the nature of the *gyn1* gene in the gynandromorphic strain? Does it have homology with *transformer*? How is imprinting regulated in *Nasonia*? We are currently trying to answer these questions. The shortly available total genome sequence of *Nasonia* [60] will be very helpful for this.

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